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14. ABSTRACT

The ROPO program allowed Dr. Majde-Cottrell over a 15-year period to contribute about 30 publications to distinguished journals and to advance our understanding of the molecular basis of viral pathogenesis. In addition to her scientific contributions, the ROPO program allowed her to maintain credibility in the scientific community, currency in the virology and immunology literature, and to continue a productive research effort upon retirement from ONR.

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FINAL TECHNICAL REPORT

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TITLE: Mechanisms of Cytokine Induction in Acute Viral Infections

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FINAL TECHNICAL REPORT

MECHANISMS OF CYTOKINE INDUCTION IN ACUTE VIRAL INFECTIONS

TECHNICAL DESCRIPTION:

The general problem investigated by this ROPO research is the molecular basis of the "flu" syndrome induced by common RNA viruses such as influenza. Specifically, we have investigated the role of viral double-stranded RNA (dsRNA), a byproduct of viral replication, in triggering the fever, somnolence and anorexia (elements of the acute phase response, or APR) characteristic of influenza viral infections, and characterizing the immune system hormones, termed cytokines, that are induced by virus to mediate these central nervous system responses.

SUMMARY OF ACCOMPLISHMENTS:

In the 15 years that Dr. Majde-Cottrell and I collaborated on this ROPO project we demonstrated the following: a) synthetic dsRNA (poly[rI•rC]) induces an APR (altered sleep, low body temperature, weight loss) indistinguishable from an acute influenza infection of the rabbit or mouse; b) viral dsRNA can be extracted from the lungs of influenza-infected mice that will induce an APR in the rabbit identical to that induced by poly[rI•rC]; c) pretreatment of rabbits with poly[rI•rC] will abolish the APR (i.e., induce a hyporesponsive state) to influenza virus in the same manner as influenza virus itself; d) live virus capable of producing dsRNA is required for a viral APR; e) synthetic viral dsRNA of a small size (108 base pairs) induces an APR in the rabbit; f) viral dsRNA leaches out of dying cells in vitro and is thus available to act on neighboring cells to induce cytokines; g) a component of the APR to influenza virus consists of excess slow wave sleep and reduced rapid eye movement sleep; the same sleep alterations are seen when animals are challenged with poly[rI•rC]; h) the type I interferon interferon-α, a cytokine made in large amounts during viral infections and induced by dsRNA and viruses, causes an acute phase response in rabbits identical to that induced by poly[rI•rC]; i) the APR induced by poly[rI•rC] introduced into the mouse lung has similar sleep and temperature elements as influenza virus.

All of the above findings are supportive of our hypothesis that viral dsRNA is an initiator of the viral APR and acts through cytokine induction. However, recent findings with a mouse strain genetically deficient in the receptor for type I interferons reveals that the APR induced by poly[rI•rC] is slightly enhanced in the absence of a response to type I interferons. This unexpected finding suggests that cytokines other than interferon-α are key regulators of the viral APR, and that type I interferons actually suppress viral symptoms. Furthermore, we observed that normal rapid eye movement (REM) sleep was substantially suppressed in the type I interferon receptor-deficient mouse, implying a role for endogenous type I interferons in normal sleep regulation. Interferons have previously been assumed to function only when a virus invades the body, and a physiological role for interferons in sleep regulation has never been suspected. This unanticipated finding is currently under investigation.

In addition to the above investigations on dsRNA, Dr. Majde-Cottrell has participated in several other projects in my laboratory: 1) the role of influenza viremia in triggering the neural responses to virus; 2) the role of growth hormone releasing hormone in regulating the sleep and temperature responses to influenza virus; 3) the role of inducible and neural nitric oxide synthases in the sleep and temperature responses to influenza virus; 4) the sleep response to the combined cytokines tumor necrosis factor-α and interferon-γ; and others. The influenza infection model she has brought to our laboratory is an important tool for investigating the molecular basis and functions of sleep—the basic mission of my laboratory.

Dr. Majde-Cottrell retired from ONR in September, 2002, but continues to collaborate on this project. The project is now supported by a grant from the National Institute for Child Health and Development.

SIGNIFICANCE OF THIS ROPO PROJECT TO THE NAVY:

Infectious diseases, many of viral origin, continue to account for extensive lost duty time in the military, especially in recruit training camps. Acute respiratory and gastrointestinal agents impair operational performance and occasionally abort missions and training programs. One possible approach to coping with performance decrements due to viral infections is to treat the disease rather than the infection. Cytokines probably mediate this disease process. Therefore an understanding of which cytokines are operating in acute viral infections may allow effective treatment of illness through appropriate cytokine antibodies or receptor blockers. Such reagents are under development for treating bacterial sepsis, and thus this investment can potentially be leveraged for viral disease therapy.

PUBLICATIONS 1988 TO 2003:

Refereed Journals:

1. Krueger, J.M., **Majde, J.A.**, Blatteis, C.M., Endsley, J., Ahokas, R.A. and Cady, A.B. (1988) Polyriboinosinic:polyribocytidylic acid (poly I:C) enhances rabbit slow-wave sleep. *Amer. J. Physiol.* 255:R748-R755.

2. Krueger, J.M. and **Majde, J.A.** (1990) Short Analytical Review: Sleep as a host defense: Its regulation by microbial products and cytokines. *Clin. Exp. Immunol.*, 57, 188-199.

3. **Majde, J.A.**, Brown, R.K., Jones, M.W., Dieffenbach, C.W., Maitra, N., Krueger, J.M., Cady, A.B., Smitka, C.W. and Maassab, H.F. (1991) Detection of toxic viral-associated double-stranded RNA (dsRNA) in influenza-infected lung. *Microb. Pathogen.* 10, 105-115.

4. Kimura-Takeuchi, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1992) Influenza virus-induced changes in rabbit sleep and acute phase responses. *Am. J. Physiol.* 263: R1115-R1121.

5. Kimura-Takeuchi, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1992) The role of double-stranded RNA in the induction of the acute phase response in an abortive influenza viral infection, *J. Infectious Dis.* 166:1266-1275.

6. **Majde, J.A.**, (1993) Microbial cell wall contaminants in peptides; a possible source of physiological artifacts. (Review), *Peptides* 14:629-632.

7. Kimura, M., **Majde, J.A.**, Toth, L.A., Opp, M.R., and Krueger, J.M. (1994) Somnogenic effects of rabbit and human recombinant interferons in rabbits. *Amer. J. Physiol.* 267:R53-R61.

8. **Majde, J.A.** (1994) An overview of cytokines and their associations with the brain. *Ann. New York Acad. Sci.,* 739:262-269.

9. Kimura, M., Toth, L.A. Agostini, H., Perera, P.Y., Vogel, S.N., Cady, A.B., **Majde, J.A.** and Krueger, J.M. (1994) Comparison of acute-phase responses induced in rabbits by lipopoly-saccharide and double-stranded RNA. *Amer. J. Physiol.,* 267:R1596-R1605.

10. Krueger, J.M. and **Majde, J.A.** (1995) Cytokines and sleep (mini-review). *Int. Arch. Allergy Immunol.* 106:97-100.

11. Krueger, J.M. and **Majde, J.A.** (1995) Microbial products and cytokines in sleep and fever regulation. *Crit. Rev. Immunol.* 14:355-379.

12. Fang, J., Sanborn, CK, Renegar, K.B., **Majde, J.A.** and Krueger, J.M. (1995) Influenza viral infections enhance sleep in mice. *Proc. Soc. Exp. Biol. Med.* 210: 242-252.

13. Fang, J., Tooley, D., Gatewood, C., Renegar, K.B., **Majde, J.A.**, and Krueger, J.M. (1996) Differential effects of total and upper airway influenza viral infection on sleep in mice. *Sleep* 19:337-342.

14. Guha-Thakurta, N. and **Majde, J.A.** (1997) Early induction of proinflammatory cytokine and type I interferon mRNAs following Newcastle disease virus, poly[rI•rC] or low dose LPS challenge of the mouse. *J. Interferon Cytokine Res.* 17:197-204.

15. **Majde, J.A.**, Guha-Thakurta, N., Chen, Z., Bredow, S. and Krueger, J.M. (1998) Spontaneous release of stable viral double-stranded RNA in the extracellular medium by influenza virus-infected MDCK epithelial cells: Implications for the viral acute phase response. *Archives Virol.* 143:2371-2380.

16. Fang, J., Bredow, S., Taishi, P., **Majde, J.A.** and Krueger, J.M. (1999) Synthetic influenza viral double-stranded RNA induces an acute-phase response in rabbits. *J. Medical Virol.* 57:198-203.

17. **Majde, J.A.** (2000) Double-stranded RNA, cytokines and the flu. *J. Interferon Cytokine Res.* 20:259-272.

18. Rehman, A., Taishi, P., Fang, J., **Majde, J.A.**, and Krueger, J.M. (2001) The cloning of a rat peptidoglycan recognition protein (PGRP) and its induction in brain by sleep deprivation. *Cytokine*, 13:8-17.

19. Kubota, T., **Majde, J.A.**, Brown, R.A. and Krueger, J.M. (2001) Tumor necrosis factor receptor fragment attenuates interferon-γ-induced non-REM sleep in rabbits. *Neuroimmunology* 119:192-198.

20. Krueger, J.M., **Majde, J.A.** and Obal, Jr., F. (2003) Sleep in host defense. *Brain Beh. Immun.* 17(1 Suppl.):41-47.

21. Alt, J.A., Obal, F, Jr., Traynor, T.R., Gardi, J., **Majde, J.A.** and Krueger, J.M. (2003) Alterations in EEG activity and sleep after influenza viral infection in GHRH receptor deficient mice. *J. Appl. Physiol.*, in press.

22. Chen, L., **Majde, J.A.** and Krueger, J.M. (2003) Spontaneous sleep in mice with targeted disruptions of neuronal or inducible nitric oxide synthase genes. *Brain Res.*, in press.

Book Chapters:

1. Krueger, J.M., Fang, J., and **Majde, J.A.** (2000) Sleep in Health and Disease. In: *Psychoneuroimmunology*, Third Edition, Vol. 1, ed. R. Ader, D.L. Felten and N. Cohen, Academic Press, San Diego, pp 667-685.

2. **Majde, J.A.** and Krueger, J.M. (2000) Neuroimmunology of sleep. In: *Biological Psychiatry*, ed. H. D'haenen, J.A. den Boer and P. Willner, John Wiley & Sons, Ltd., London, pp 1247-1257.

Abstracts:

1. Endsley, J., Ahokas, R.A., **Majde, J.A.**, Blatteis, C.M. and Krueger, J.M. (1987) Enhancement of slow-wave sleep (SWS) by polyriboinosinic:polyribocytidylic acid (poly I:C). *Fed. Proc.* 46:1128.

2. Cady, A.B., Brown, R.K., Jones, M.W., **Majde, J.A.** and Krueger, J.M. (1989) RNA from influenza-infected lungs increases slow-wave sleep and body temperature while decreasing REM sleep. *Fed. Proc.* 3:A678.

3. **Majde, J.A.**, Cady, A.B., Brown, R.K., Jones, M.W. and Krueger, J.M. (1989) RNase-resistant RNA from influenza-infected lungs alters body temperature and sleep. *Cytokine* 1:112.

4. **Majde, J.A.**, Dieffenbach, C.W., Havell, E.A., Kluger, M.J., and Maassab, H.F. (1990) Effects of tumor necrosis factor-α (TNF-α) antibody on acute PR8 influenza in the mouse. *Abstracts VIIIth Intern. Congress Virology, Berlin,* P63-002, p. 411.

5. **Majde, J.A.**, Kluger, M., Maassab, H., Brown, R., Jones, M., Maitra, N., and Dieffenbach, C. (1990) Cytokine mRNA expression and presence in the circulation during acute influenza in the mouse. *J. Leukocyte Biol. Suppl.*, p. 81.

6. Kimura-Takeuchi, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1991) Hypnogenic tolerance to influenza virus is induced by double-stranded RNA in rabbits. *Sleep Res.* 20A:428.

7. Kimura-Takeuchi, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1991) Influenza virus and poly I:C enhance antiviral activity in rabbit sera, but not in rabbits tolerized to the virus. *Society for Neuroscience Absts.* 17:832.

8. **Majde, J.A.**, Kimura-Takeuchi, M. and Krueger, J. (1991) Physiological hyporesponiveness to influenza virus is induced by dsRNA and associated with absence of antiviral activity. *Cytokine* 3:511.

9. Kluger, M.J., Conn, C.A., McClellan, J.L., Maassab, H., Smitka, C. and **Majde, J.** (1991) TNFα levels, physiological responses and behavioral changes associated with influenza viral pneumonitis. *Cytokine* 3:510.

10. Kimura-Takeuchi, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1991) Tolerance to virally-induced changes in sleep of rabbits. *Sleep Res.* 20:268.

11. **Majde, J.A.**, (1992) CNS mediation of acute extraneural viral infections, or, how the flu makes you sick. *Winter Conference on Brain Research, Steamboat Springs, CO*, Specialized Panel 71.

12. Kimura-Takeuchi, M, **Majde, J.A.**, Toth, L.A. and Krueger, J.M (1992) Somnogenic action of beta interferon: effects in influenza virus-tolerant rabbits. *J. Sleep Res.* 1, Suppl. 1:115

13. Kimura-Takeuchi, **Majde, J.A.**, Toth, L.A., Opp, M.R. and Krueger, J.M. (1992) Human and rabbit interferons induce sleep in rabbits. *22nd Ann. Meet. Soc. Neuroscience Abst.* 512.6, p. 1227.

14. Dieffenbach, C., Agostini, H., Guha Thakurta, N., **Majde, J.**, and Vogel, S. (1992) Comparison of cytokine induction by lipopolysaccharide (LPS) and the double-stranded RNA poly[rI:rC]. *8th Internatl. Congr. Immunol. (Budapest) Abstracts*, W-64-9, p. 400.

15. **Majde, J.A.**, Guha Thakurta, N. and Dieffenbach, C.W. (1992) Induction of interleukin-1ß (IL-1ß) mRNA as well as IL-1α mRNA in adrenal by nicotine. *Hans Selye Symp. Neuroendocrin. Stress (Budapest) Abstracts,* p. 43.

16. Kimura-Takeuchi, M, **Majde, J.A.**, Toth, L.A., Opp, M.R. and Krueger, J.M. (1993) Somnogenic actions of human recombinant interferon-ß and rabbit interferon-α/ß in rabbits. *Sleep Res.* 22:437.

17. Kimura-Takeuchi, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1993) Effects of recombinant human interferons on sleep in rabbits. *Sleep Res.*, 22:36.

18. Guha-Thakurta, N., Dunn, A.J., Jenkins, F.J. and **Majde, J.A.** (1993) Rapid induction of cytokine mRNAs by virus. *Abstracts First ONR Workshop on Immunophysiology (Washington, D.C.)* p. 16-17.

19. Guha-Thakurta, N., Dunn, A.J., Jenkins, F.J. and **Majde, J.A.** (1993) Very early induction of cytokine mRNAs in the mouse spleen by Newcastle disease virus. *Amer. Soc. Virology Abstracts, Davis, CA, 10-14 July 1993.*

20. **Majde, J.A.**, Jenkins, F.J., and Guha-Thakurta, N. (1993) Very early induction of cytokine mRNAs by RNA viruses, allantoic fluid and dsRNA in the mouse spleen. *Lymphokine Cytokine Res.* 12:361.

21. Kimura, M., Toth, L.A., Cady, A.B., **Majde, J.A.**, and Krueger, J.M. (1993) Acute-phase responses of rabbits to endotoxin and poly I:C. *23rd Ann. Meeting Absts. Soc. Neurosci.*, p. 97.

22. Fang, J., Sanborn, C.K. **Majde, J.A.**, and Krueger, J.M. (1994) Sleep changes induced by influenza virus infection in mice. *Sleep Res.* 23:358.

23. Kimura, M., **Majde, J.A.**, Toth, L.A. and Krueger, J.M. (1994) Effects of recombinant human interferon-α and -β on sleep in rabbits. *Jap. J. Psychiatry Neurol.* 48:181.

24. Fang, J., Renegar, K.B., **Majde, J.A.** and Krueger, J.M. (1994) Influenza viral infection induces sleep changes in mice. *24th Ann. Meeting Abst. Soc. Neurosci.* 20:157.

25. Bredow, S., Fang, J., Guha-Thakurta, N., **Majde, J.A.** and Krueger, J.M. (1995) Synthesis of an influenza double-stranded RNA oligomer that induces fever and sleep in rabbits. *Sleep Res.* 24A:101.

26. **Majde, J.A.**, Guha-Thakurta, N., Bredow, S., and Krueger, J.M. (1995) Evidence for a role for double-stranded RNA in the viral acute phase response (flu syndrome). *Molecular Pathogenesis of Viruses, Mt. Sinai School of Medicine, New York, NY Abstract 37.*

27. Guha-Thakurta, N., **Majde, J.A.**, Bredow, S. and Krueger, J.M. (1996) Characterization of viral RNA in supernates of influenza-infected MDCK cells -- detection of viral dsRNA. *Amer. Soc. Virology Abstracts, London, Ontario, Canada, 13-17 July 1996*, Abstract P17-1, p. 179.

28. **Majde, J.A.** and Krueger, J.M. (1998) Investigations of the role of double-stranded RNA in the influenza acute phase response (flu syndrome). *International Symposium on Influenza and Other Respiratory Viruses, 4-6 December 1998,* Abstract P8.

29. **Majde, J.A.**, Rehman, A. Ye, X. and Krueger, J.M. (1999) Expression of the influenza viral acute phase response precedes extrapulmonary dissemination of virus. *Amer. Soc. Virology Abstracts, Univ. of Massachusetts, Amherst, MA, 10-14 July 1999*, Abstract P16-14, p. 171.

30. Rehman, A., **Majde, J.A.**, and Krueger, J.M. (1999) Peptidoglycan recognition protein (PGRP) mRNA levels are not induced during the acute phase response to influenza virus. *The Physiologist* 42:A-9.

31. Chen, L., **Majde, J.A.**, Alt, J., Kubota, T. and Krueger, J.M. (2001) The differential sleep response of inducible NOS knockout mice to influenza infection. *Soc. Neurosci. Abst.*

32. Chen, L., Fang, J., **Majde, J.A.**, and Krueger, J.M. (2001) Impairment of the sleep responses to influenza infection in mice with a defective GHRH-receptor. *Sleep* 24:A144-A145.

33. Chen, L., Fang, J., **Majde, J.A.**, and Krueger, J.M. (2001) Effects of IFN-gamma and TNF-alpha on spontaneous sleep in mice. *Sleep* 24:A145.

34. Traynor, T.R., Alt, J.A., Obal, F., Jr., **Majde, J.A.**, and Krueger, J.M. (2002) Sleep and body temperature in mice lacking a functional GHRH receptor. *Sleep*, in press.

35. Alt, J.A., Obal, F., Jr., **Majde, J.A.** and Krueger, J.M. (2002) GH replacement fails to normalize sleep responses to influenza viral infection in GHRH-receptor deficient mice. *Sleep*, in press.

36. Chen, L., **Majde, J.A.** and Krueger, J.M. (2002) Spontaneous sleep in neuronal and inducible nitric oxide synthase knockout mice. *Sleep*, in press.